

Review

Ramey, R. R., H.-P. Liu, L. M. Carpenter, and C. W. Epps. Testing the uniqueness of *Z. h. intermedius* relative to *Z. h. campestris*.

This study examines skull morphometric variation and mtDNA sequences to determine whether *Zapus hudsonius intermedius* is distinctly different from a parapatric subspecies, *Z. h. campestris*. The morphometric data are treated with discriminant function analysis. The sequence data are used to construct a phylogenetic tree, to apportion variation within and between subspecies, and also to estimate the migration rates among these three subspecies, plus two other subspecies, *Z. H. pallidus* and *Z. h. luteus*. Thus, there are four products from this study—the discriminant function analyses, the phylogenetic tree, analysis of molecular variance, and migration rates among subspecies.

The data appear to be solid. They used nine skull measurements, and the sample sizes were good; the full analysis included measurements on 105 skulls. The authors have extensive experience with sequencing, interpreting amova, and constructing phylogenetic trees.

I think that a dedicated skeptic could raise objections about any one of the four products or types of analyses presented here. For example, I believe it is useful to be skeptical about estimates of effective population size and migration extracted from genetic data; those analyses rely on quite a few assumptions. Or, one might point out that a few of the haplotypes within the phylogenetic tree appear in the “wrong” clade. However, I note that all four analyses are pointing to the same conclusion: *Z. h. preblei* plus *Z. h. campestris* are similar to *Z. h. intermedius*. These three subspecies are quite distinct (based on DNA sequences) from two other subspecies, *Z. h. pallidus* and *Z. h. luteus*.

Skull shape, clustering of mitochondrial DNA haplotypes, analysis of variance, and estimates of gene flow all indicate that *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* are similar and are exchanging genes at a significant rate (or were in the recent past).

The estimate of M for *campestris* and *intermedius* is in the range 0.58 to 5.86. M is defined here as $2N_e m$, where N_e is the estimate for effective population size and m is the migration rate; M is twice the number of individuals moving between subspecies per generation. Evolutionary geneticists generally use the rule of thumb that migration of more than one individual between populations per generation will keep those populations from diverging via genetic drift. This relative high number of migrants per generation is consistent with no morphometric differences between *campestris* and *intermedius*, and both of these results are consistent with a low proportion of variation between subspecies, and with sharing and similarity of haplotypes.

With respect to the specific questions posed in the invitation to review:

- 1) I believe that the data and the treatment of the data are appropriate to address the questions. I think that reciprocal monophyly is too restrictive for defining subspecies; I agree that apportionment of variation within and among subspecies is more practical.
- 2) I agree that the authors found no data to support the recognition of separate subspecies (*preblei*, *campestris*, *intermedius*) but they do have data to indicate that this clade is reasonably distinct for the clade containing *pallidus* and *luteus*.
- 3) Yes, given the data presented here and in the previous paper, I believe that *Z. h. preblei* is a subset of the variation within *Z. h. campestris*.
- 4) It is possible that there are nuclear genes that adapt these different subspecies to their local habitats. This is the sort of hypothesis that cannot be rejected until we have examined every gene in the nuclear genome. I think this is useful as a caveat or heuristic caution, but I think it is extremely unlikely.
- 5) To push this further, collect morphometric data from *pallidus* and *luteus*, to determine whether the morphometric data are concordant the topology of the phylogenetic tree.
- 6) No. See 2 above.

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